THE EFFECTS OF DIFFERENT DIETARY FIBRE LEVELS ON METHANE PRODUCTION AND GROWTH PERFORMANCE OF BONSMARA AND NGUNI STEERS

by

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List of abbreviations

ADF : Acid detergent fibre

ADG : Average daily gain

ADL : Acid detergent lignin

ANOVA : Analysis of Variance

API : Animal Production Institute

ARC : Agricultural Research Council

CP : Crude protein

CF : Crude fibre

DFI : Daily feed intake

DMI : Dry matter intake

DNA : Deoxyribonucleic acid

EE : Ether extract

FCR : Feed conversion ratio

GE : Gross energy

GHG : Greenhouse gases

IVDMD : In vitro dry matter digestibility

IVGPT : In vitro gas production technique

LMD : Laser methane detector

LSD : Least significant difference

NDF : Nutrient detergent fibre

OM : Organic matter

PCR : Polymerase chain reaction

RTMC : Rumen total microbial count

Declaration

I, Sanele Thabani Jiyana, declare that, this dissertation, which I hereby submit for Master of Science in Agriculture at University of South Africa is my own original work; and that all sources used and quoted have been duly acknowledged by means of complete references; and comply with the Code of Academic Integrity, as well as procedures, rules and regulations of University of South Africa; and has not been submitted before to any other higher learning institution by myself or any other person, in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

S.T. Jiyana

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Signature Date

Dedication

This dissertation is dedicated to my daughter Miss Enhle Jiyana, who sometimes had to wake up in the middle of cold nights and offer to be with me when I was writing this dissertation.

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S.T. Jiyana, M.M. Ratsaka, K.R. Mbatha & M.K. Mkhwanazi. 2015. Comparing enteric methane emissions and relative rumen microbial counts from beef steers fed diets with different fibre and energy contents. South African Society of Animal Science. 48th Congress. 20 – 23 September. Empangeni, Kwa-Zulu Natal, South Africa.

Abstract

The aim of the study was to determine the effects of different dietary fibre levels on methane production and growth performance of Bonsmara and Nguni steers. Nine Bonsmara and nine Nguni male weaners aged 8 – 9 months were used for the study. On arrival, animals were fed *Eragrostis curvula* hay on *ad libitum* basis for the first 14 days as part of adaptation to the feedlot environment. Animals were gradually adapted to treatments to prevent metabolic disorders. The study was conducted as a 3 x 3 x 2 factorial experiment. Treatments were allocated in a completely randomised design. Data were submitted to analysis of variance (ANOVA). Student's t-LSD was calculated at the 5 % level ($P < 0.05$) to compare treatment means for significant effects. Feeding a diet that is low in crude fibre content had significantly increased ($P < 0.05$) growth performance in both breeds. Bonsmara steers had significantly higher growth performance ($P < 0.0001$) across all treatment diets compared to Nguni steers. High rumen total microbial count was observed in animals that received diets with low crude fibre. Reducing dietary crude fibre resulted in reduced methane production. Low dietary fibre showed low rumen *archaea* counts. There was a positive correlation between rumen pH and methane emission for both Bonsmara ($R^2 = 0.9105$) and Nguni ($R^2 = 0.9517$) steers. However, a negative correlation was observed between rumen total microbial count and feed conversion ratio for both Bonsmara ($R^2 = 0.8286$) and Nguni ($R^2 = 0.7208$) steers. The low rumen *archaea* counts were detected from the Bonsmara. Feeding diets with low fibre levels is recommended for improving growth performance and reducing methane production for Bonsmara and Nguni steers.

Chapter 1

1 Introduction

1.1 Background

Ruminants are capable of using fibre that cannot be utilised directly by non-ruminants (Morgavi *et al.*, 2010). However, a negative aspect of this process is the creation of greenhouse gases (GHG), especially ruminal methane. Methanogens are the methane-producing bacteria in the rumen (Shibata & Terada, 2010). They use hydrogen to decrease carbon dioxide (CO_2) and produce energy for growth, with methane gas as one of the products. If this hydrogen is not utilised, it can prevent rumen metabolism (Hook, Wright & McBridge, 2010). The amount of methane produced depends on quantity and quality of feed consumed by the animals (Singh, 2010). Ruminants are identified as the major source of methane gas (NRC, 2002).

According to Wright *et al.* (2004), the contribution of enteric methane to the build-up of GHG and its ability to waste feed energy is a worldwide concern. Agriculture system is accountable for 5 to 10 % of the global GHG emissions (Scholtz *et al*., 2013). Enteric fermentation is responsible for 60 % contributed by livestock (Grobler *et al.*, 2014). Beef cattle are identified as the major source of enteric methane production, compared to other domestic ruminants. McMichael *et al*. (2007) showed that approximately 65 % of methane produced by domestic ruminants is from beef cattle, 22 % is from dairy cattle and only 13 % is from sheep and goats.

Enteric methane production consumes 2 to 12 % of the dietary energy meant for animal production (Ramin & Huhtanen, 2013) depending on the feed quality. Furthermore, the components of the diet such as carbohydrates are vital for methane production, since they reduce ruminal pH and eventually change the microbiota. Ellis *et al*. (2007) predicted that

enteric methane production is related to dry matter intake (DMI), acid detergent lignin (ADL), and neutral detergent fibre (NDF).

The starch component of a diet promotes propionate production, by a shift to amylolytic bacteria and decrease of ruminal pH, resulting in reduced methane production (Popova, Morgavi & Martin, 2012). Minimising forage particles prior to feeding decreases methane production (Hook, Wright & McBridge, 2010). The improved passage rate of milled forage through the gut limits the production period of methane in the rumen. Increased feed passage rate also shifts methanogenesis to the hindgut thus, reducing the ruminal methane outputs (Hindrichsen *et al*., 2006).

According to Singh (2010) replacing plant fibre with starch induces a change of volatile fatty acids (VFAs) from acetates to propionates, resulting in less hydrogen production and eventually the need for methanogens within a rumen is reduced. Yan *et al*. (2000) reported that a high proportion of concentrates in a diet causes a reduction of methane production. Feeding steam flaked corn to cattle reduces production of enteric methane nearly 20 %, due to more efficient digestion of starch in the rumen (Hales, Cole & MacDonald, 2012). Inclusion of ionophores such as monensin in ruminant rations improves the efficacy of cattle and decreases production of methane (Guan *et al*., 2006). Supplementary with lipid decreases production of ruminal methane (Beauchemin *et al.*, 2008). However, due to negative effects of lipid supplementation on rumen fermentation, its levels should be limited to below 4 % of dry matter. High level of oil in diets lowers nutrient digestibility (Martin *et al*., 2008).

Driller's grains may reduce enteric methane production, when used in diet with low fat content (Hales, Cole & MacDonald, 2012). However, the same driller's grains will not reduce enteric methane production if fed to animals in equal fat diets. Chloroform (halogenated compound) has inhibitory impacts on enteric methane production (Hook, Wright & McBridge,

2010). Feeding of condensed tannins, dicarboxylic acids, yeast cultures, saponins and the use of vaccines can reduce enteric methane production (Beauchemin *et al.*, 2008).

Cattle emit 89 % of the enteric methane produced from a rumen through the nostrils and mouth (Broucek, 2014). Chagunda, Ross & Roberts (2009) demonstrated that results produced by the use of laser methane detector (LMD) are credible. The laser methane detector is pointed on the muzzle, during the measurement of methane emissions (Chagunda & Yan, 2011). The *in vitro* gas production technique (IVGPT) is one of the best methods that are used to measure methane produced by ruminants (Rymer *et al.*, 2005).

1.2 Problem statement

There is a concern about the impacts of enteric methane production on global warming. Different species and breeds of both domesticated and non-domesticated ruminants play variable roles towards greenhouse gas production (Scholtz *et al*., 2014). However, it is not known exactly which breed or species will contribute more to greenhouse gas production. The diet that the animals consume is a critical factor to the end product of the digestion process (Singh, 2010). Scientists have investigated various nutritional strategies to reduce enteric methane production (Hook, Wright & McBridge, 2010). Supplemental fats, starch, ionophores, concentrates, halogenated compounds, condensed tannins, probiotic yeast products and direct-fed microbials are some of nutritional interventions that have been applied to reduce methane production (Beauchemin *et al.*, 2008; Johnson & Johnson, 1995; Yan *et al*., 2000; Guan *et al.*, 2006, Vohra *et al*., 2016; Jeyanathan *et al*., 2014). However, most of these nutritional interventions are very expensive and thus not practicable to the most of livestock farmers, especially communal farmers who are poorly resourced.

1.3 Justification of the study

Global warming poses changes, threats and challenges to both farming society and scientific communities. Climate change should be understood and addressed urgently for survival, livelihoods and sustenance of the environment. Agricultural production should be increased without exacerbating the negative effects of global warming such as emission of greenhouse gases. A satisfactory solution to greenhouse gas emission is still not yet found. The scientists in animal production should formulate methods to reduce the methane production without changing performance of animals especial beef cattle. Proposed intervention should be affordable and easily accessible, to accommodate the entire beef cattle farmers, including communal farmers. Most of these farmers are usually poorly resourced. The results from this study will provide optimal levels of fibre in a feedlot diet that can reduce methane production and still maintain optimal growth performance.

1.4 Objectives

The main objective

To determine optimal level of fibre in a feedlot diet at which methane production can be low without reducing growth performance in Bonsmara and Nguni steers.

The specific objectives

- To determine the effects of different dietary fibre levels on growth performance of Bonsmara and Nguni steers.
- To ascertain the effects of different dietary fibre levels on methane emission, rumen pH levels, rumen total microbial count and rumen methanogenic *archaea* count from Bonsmara and Nguni steers.

1.4 Hypotheses

The main hypothesis

It was hypothesised that different dietary fibre levels in diets of Bonsmara and Nguni steers will not reduce methane production or improve their growth performance

The specific hypotheses

- Different dietary fibre levels will not improve growth performance of Bonsmara and Nguni steers.
- Different dietary fibre levels will not reduce enteric methane emissions, rumen pH level, rumen total microbial count and rumen methanogenic *archaea* counts of Bonsmara and Nguni steers.

Chapter 2

2 Literature review

2.1 The role of ruminant nutrition to the society

African rural households rely on livestock for meat, milk, hides and income (Dovie *et al*., 2006). This might bring income for the poorly resourced rural households to reduce their living expenses. Beef meat is a source of nutrients such as protein that is essential for body cells and tissues development, plus selenium that is critical to the human anti-oxidant defence system (Cox, 2011). Beef meat similarly provides vitamin B12 that is essential for human body development, vitamin B6 that is necessary for absorption of amino acids, iron that is essential for red blood cells production and zinc that is necessary for strong immune system (Cox, 2011). Ruminants play a primary part in supporting human culture, they are also used as draft animals and for rituals (Henderson *et al*., 2015). Ruminants are capable of eating fibrous feedstuffs that are low in protein and energy but convert them into high quality protein (Rodríguez, Sosa & Rodríguez, 2007). This is due to the unique digestive system that ruminants possess.

2.2 The effects of dietary fibre level on chewing period and rumen pH

Saliva secreted during chewing contains natural buffers such as bicarbonate, sodium and potassium, which stabilise the rumen pH (Hernández *et al*., 2014). The actual volume of saliva secreted by ruminants depends on type of feed, feeding time and ruminating period (González *et al*., 2012). Thus, ruminants on high fibre diets or pure grazing hardly experience the excessive lowered rumen pH, since they spend more time on chewing the high fibre diet, resulting in more secretion of natural buffers from saliva (Hernández *et al*., 2014), which consequently results in properly balanced rumen pH (Brown, Ponce & Pulikanti, 2006). The dietary buffers are added in high energy or grain diets to obtain neutralisation of rumen acid

(Hernández *et al*., 2014). In large ruminants, a rumen has a volume of approximately 95 L and contains millions of various protozoa and bacteria (Brooker *et al*., 2008). These microorganisms secrete enzymes that assist in digestion of plant material into VFAs, amino acids and fatty acids for the benefit of the host.

2.3 Effects of ruminal pH on enteric methane production and rumen microbiota

Fermentation of high fibre diet results in an accepted balance in microbial activity from ingestion until the excretion point (Brown, Ponce & Pulikanti, 2006) and the rumen pH that is within a normal range (6 - 7). Ingestion of highly fermentable carbohydrates such as starch diet results in high rumen microbial growth rate and fermentation characteristics (Nagaraja & Lechtenberg, 2007). This leads to high production of VFAs and lactic acid within a rumen. The VFAs provide energy to the animal, because they are an easy form by which carbohydrates absorbed from rumen to the animal blood system (Hünerberg *et al*., 2015). Accumulation of lactic acid in the rumen results in a shift in microbial density from gram-negative prevalence to gram-positive lactic acid producers. This leads to excessively low ruminal pH, due to high rumen acidity.

Methanogens, like most of other rumen microbes, are very unstable to low levels of ruminal pH (Popova, Morgavi & Martin, 2012). Methanogens do not die at rumen pH below 6.0 but they enter metabolic stasis (Hünerberg *et al*., 2015). Furthermore, when pH of the same rumen fluid is reversed to be above 6.0, methane production starts again (Hünerberg *et al*., 2015). Feeding cattle with diets rich in soluble starch or carbohydrates might result to ruminal pH below 6.0 for prolonged periods (Hünerberg *et al*., 2015). Feeding diet that consists high-grain could be considered as one of viable strategies to decrease ruminal pH (Beauchemin *et al*., 2008) reducing enteric production of methane by ruminants.

2.4 Greenhouse gases produced by ruminants through enteric fermentation

Methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) are GHGs that are produced by ruminants as by-products of enteric fermentation. In 2006, the Food and Agriculture Organisation, Livestock's Long Shadow, did a mistake by indicating that 18 % of the world's GHG emission is from livestock (Steinfeld *et al*., 2006). This information has been proved to be an overestimation, since it includes deforestation and other indirect contributions (Pitesky *et al*., 2009). The most recent figure is 5 to 10 % of the global GHG emissions (Scholtz *et al*., 2013). Enteric fermentation is responsible for 60 % of the 5 to 10 % contributed by livestock (Grobler *et al.*, 2014). This makes enteric methane mitigation a critical process that needs to be prioritised in terms of animal production research.

2.5 Methane as a major contributor towards global warming

Rumen methanogens use enteric hydrogen to reduce CO_2 formed as products of microbial feed digestion process and produce energy for growth, with methane gas as end product (Hook, Wright & McBridge, 2010). If hydrogen is not used, it can prevent rumen metabolism. According to Wright, Aukland and Lynn (2004) the contribution of enteric methane to the accumulation of GHG and wasting feed energy is a worldwide concern. Ruminants are the principal source of methane gas (NRC, 2002).

The potential of methane on global warming is 23 times more than that of CO_2 (Scholtz *et al*., 2013). Enteric methane production wastes 2 to 12 % of the dietary energy meant for animal production, which can be 150 to 300 L per day. Furthermore, this contributes approximately 16 to 20 % of global atmospheric methane (Ramin & Huhtanen, 2013) depending on the feed quality and quantity. Thus, it is crucial for beef farmers to ensure that the chemical composition of the forage and feed offered to ruminants correlates with the nutrient requirements of the physiological needs of animal.

2.6 Dietary fibre digestion in ruminants

The fibre component of feed has a major impact on digestibility, and both chemical composition and amount of dietary fibre are crucial (Smith, 2008). Digestibility of dietary fibre refers to the proportion of ingested feed that is not excreted as faeces or urine, thus assumed to be absorbed by the animal (McDonald *et al*., 2002). Dietary fibre components include; crude fibre, acid detergent fibre, acid detergent lignin, and neutral detergent fibre. Several rumen microbial populations ferment dietary fibre to provide volatile fatty acids and proteins to the host animal (Varga & Kolver, 1997). The extent of fibre fermentation depends on microbial accessibility to substrate, chemical composition of the diet, physical characteristics of the diet and rumen fermentation kinetics (Smith, 2008).

2.7 Factors affecting enteric methane production from ruminants

2.7.1 Rumen microbial community

Rumen microbes can be grouped into various groups such as cellulolytic, amylolytic and proteolytic, which degrade a wide variety of feed components or further metabolise some of the products formed by other microbes (Henderson *et al*., 2015). Similarly, methanogens metabolise hydrogen formed by fermentative microbes to produce methane (Broucek, 2014). The methanogen populations present in the rumen depend on the diet consumed by the host animal (Kamra, 2005). Whitford *et al*. (2001) found *Methanobrevibacter ruminantium* as the largest group of methanogens in lactating dairy cows fed total mixed rations, followed by *Methanosphaera stadtmanae*.

On the other hand, Wright *et al*. (2007) found *Methanobrevibacter spp*. from a clone library of the rumen fluid of feedlot cattle fed on a predominantly corn diet. Methanogen strain is also associated with the feed efficiency of a diet (Whitford *et al*., 2001). This emphasises the fact that feed efficiency has more influence than animal species on rumen microbial community

composition (Henderson *et al*., 2015). There is a strong symbiotic association between methanogens and protozoa, which provides methanogens with the hydrogen produced by protozoa to reduce carbon dioxide to methane (Machmüller, Soliva & Kreuzer, 2003).

2.7.2 Feed quality and chemical composition

Fermentable carbohydrates and nitrogen compounds are required for proper growth of rumen microorganisms, rumen synthesis of protein, minerals and vitamins (Sath, 2012). The production of enteric methane is predictable based on NDF, DMI and ADL concentrations of the diet (Ellis *et al*., 2007). High dietary concentrate proportion results in reduction of methane emission as a part of energy intake (Yan *et al*., 2000). Therefore, animals should be fed only high-quality diets or forage to reduce enteric methane production.

2.7.3 Feed processing prior to feeding

Grinding of forage or feed before it is fed to cattle decreased methane production, by increasing the digestion rate thus reducing the time for production of methane (Hook, Wright & McBridge, 2010). Increasing the quantity of rapidly fermentable carbohydrate enhances the passage rate from the rumen (Hindrichsen *et al*., 2006). According to Singh (2010) replacing plant fibre with starch encourages a change of VFAs production from acetate to propionate, resulting in reduced hydrogen production. Consequently, the need for methanogenesis is limited. Feeding grasses or lucerne as silage decreases enteric methane emissions compared to dry hay (Beauchemin *et al*., 2008). Ionophores are feed additives included in a diet to enhance the nutritional efficiency of feedlot cattle (Guan *et al*. 2006). However, their inclusion in ruminant diets is also important in decreasing enteric production methane by about 10 to 20 % (Guan *et al*., 2006).

2.8 Dietary mitigation of enteric methane production in ruminants

Supplementing diet with fat decreases ruminal methane production by 5 to 20 % in finishing diets (Beauchemin *et al*., 2008). Hales, Cole & MacDonald (2012) stated that when distiller's grains are included in diets with low fat levels, they tend to drop enteric methane emission. Nevertheless, these grains do not affect enteric methane production when fed in the same amount as fat diets.

Condensed tannins (CT) tend to inhibit methanogens, through a decline of hydrogen (Tavendale *et al*., 2005). The CT in *Lespedeza cuneata* fed to goats resulted in reduced methane production by 57 % (Puchala *et al*., 2005). Production of methane was reduced by 13 % in sheep fed 41 g of CT of *Acacia mearnsii* per kg of dry matter (Carulla *et al*., 2005). The plant species that contained CT reduced methane emission by 24 % when fed to lambs (Tiemann *et al*., 2008). However, CT extracts from sorghum silage (de Oliveira *et al*., 2007) and *Schinopsis quebrachocolorado* (Beauchemin, *et al*., 2007) fed to cattle did not reduce methanogenesis.

2.9 Methods used to measure enteric methane produced by ruminants

Several techniques were developed to determine the amount of CH_4 produced by ruminants through enteric fermentation (Hammond *et al*., 2016). The ruminants emit enteric methane in three ways (Ricci, *et al*. 2014). Firstly, methane produced from the rumen and lower gastrointestinal gut is absorbed into the blood stream and exhaled from the lungs through the nose. Secondly, methane is discharged directly from the rumen by belching or orally. Lastly, methane is discharged from the hindgut via the rectum. These processes enable the selection of best technique that will target an organ for enteric methane assessment. Approximately 2 % of enteric methane is emitted via rectum, while nearly 87 % of is released nasally and orally (Hammond *et al*., 2016). The remaining 11 % of enteric methane is soaked up into the portal vein and disappear (Reynolds *et al*., 2013).

2.9.1 Laser methane detector technique

A hand-held laser methane detector (LMD) is used to measure methane concentration between the nose and mouth of animals (Ricci *et al*., 2014). The methane can be measured by a portable LMD held at 1 to 3 m from the animal and for 2 to 4 continuous minutes (Hammond *et al*., 2016). The data consist of sequences of peaks that represent the respiratory cycle of an animal. Only peaks that represent the increase in CH_4 concentration due to eructation or exhalation are utilised for analyses (Ricci *et al*., 2014). According to Ricci *et al*. (2014), data collected using LMD must be separated into respired and eructated methane, to be compared with results from respiration chambers and to improve the sensitivity of the technique. The LMD technique enables measurements that are more frequent while animals are in their normal state (Hammond *et al*., 2016) provided they do not move during CH_4 recording.

2.9.2 Respiration chamber and enclosure technique

A wide range of open-circuit respiration chambers that house the whole animal, are the most used to measure enteric methane production (Hammond *et al*., 2016). The air distributed through the respiration chamber and around the animal blend with incoming air and emitted methane in the capacity, when collecting data on inward and outward air for gas analysis. Maintaining average temperature, pressure and humidity conditions within a respiratory chamber is as highly significant to ensure accuracy of methane concentrations being measured (Herd *et al*., 2014). The CH_4 contained in the chamber at initial and termination of measurements should be accounted for. These measurements are done over a period of 2 to 7 conservative days (Schwarm *et al*., 2015), depending on the objectives of study and availability of resources.

2.9.3 Sulphur hexafluoride (SF_6) tracer technique

The sulphur hexafluoride (SF_6) tracer technique is suitable for both penned and free-range animals (Hammond *et al*., 2016). This technique depends on the placement of infusion tube with identified SF_6 gas release rate into the reticulorumen. Data of exhaled air are constantly collected using tubes placed near the nose and the mouth of animal and connected to a pre-decamped container (Williams *et al*., 2011).

2.9.4 Automated head chamber (GreenFeed) technique

This technique measures methane emission by using a mixture of an extractor fan and sensors that encourage recorded airflow to move past the head of an animal while allowing released gas to be collected together and tested (Hammond *et al*., 2016). The animal is enticed by a supplemental feed offered in a head chamber to enter the GreenFeed unit. During eating, the measurement of methane emission is conducted (Huhtanen *et al*., 2015).

2.9.5 Carbon dioxide as a tracer to estimate daily methane emission

Most of CO_2 is formed through transitional metabolism of the animal but enteric fermentation plays a bigger share (Hammond *et al*., 2016). Carbon dioxide emission is based on estimated energy metabolism, heat production, energy metabolism, carbon balance and respiratory quotient (Madsen *et al*., 2010). Estimation of produced and exhaled amount of CO_2 permits measureable CH_4 emission from synchronized results of CH_4 and CO_2 levels in exhaled gas samples (Hammond *et al*., 2016). A positive affiliation between predicted and observed CO_2 production of lactating dairy cows was detected (Hellwing *et al*., 2013).

2.9.6 *In vitro* gas production technique (IVGPT)

During *in vitro* gas production technique (IVGPT), experimental diets are incubated at 39°C with a combination of rumen fluid, minerals and buffer for a period of 6; 12; 24; 48; 72; 96 or 144 hours, to ferment feed (Storm *et al*., 2012). The incubation period depends on the fermentation kinetics of the specific dietary treatment and the objective of the study. The disadvantages of this technique are that it only simulates the ruminal fermentation of feed, but it does not completely measure emission and digestibility by the animal (Storm *et al*., 2012). However, with IVGPT it is easy to regulate fermentation environments like pH (Hammond *et al*., 2016).

2.10 Summary

It is highly significant to prioritise the reduction of methane emitted by livestock. Methane is not the only GHG emitted by ruminants, however, its negative impact on global warming is 23 times than that of CO_2 (Scholtz *et al*., 2013). This makes enteric methane production a critical issue. Several techniques have been developed and applied to quantify enteric methane emitted by ruminants (Hammond *et al*., 2016). An *in vitro* gas production technique is applied to guarantee that mitigation of methane production does not compromise nutrient requirements of animals using feedstuffs with poor fermentation kinetics (Storm *et al*., 2012).

The current study evaluated the optimal level of fibre in a feedlot diet at which methane production can be low without reducing growth performance in Bonsmara and Nguni steers. Easily accessible and affordable feed ingredients were used during formulation of treatment diets. The findings of the current study could be applicable to beef farmers. Reducing unnecessary loss of 2 – 12 % dietary energy (Ramin & Huhtanen, 2013) associated with enteric methane production while maintaining optimal growth performance would really increase profit margins for beef farmers.

Chapter 3

3 Materials and methods

3.1 Study area

This study was conducted at the Beef Cattle Feedlot and the Nutritional Microbiology Laboratory sections of the ARC – API in Pretoria, Gauteng, South Africa (25º 53' 59.6" S and 28º 12' 51.6" E). The area is characterized by an ambient temperature range of 18 to 29º C during summer and between 5 and 20º C during winter. The experiment was conducted for 90 consecutive days (September to November 2015), with animals placed in single feeding pens.

3.2 Experimental animals

Nine Bonsmara and nine Nguni male weaners, aged 8 - 9 months old, were used in the study. The average initial live body weights (kg) were 225 ± 10.04 (Mean ± SD) for Bonsmara and 215 ± 10.02 kg for Nguni breed. The Bonsmara weaners were sourced from ARC Roodeplaat farm and the Nguni weaners were sourced from ARC Loskop farm. On arrival to the feedlot, animals were processed according to the method prescribed by Chester-Jones & DiCostanzo (2012) including quarantine. Animals were vaccinated against common feedlot diseases, dewormed, injected with growth stimulants and dipped for external parasites. The steers were handled according to the policies and procedures of the ARC-API, Animal Ethics Committee. Approvals to conduct this study were obtained from the animal ethics committees, ARC-API (Reference number: APIEC15/047) and UNISA, College of Agriculture and Environmental Sciences (Reference number: 2015/CAES/130).

3.3 Housing

Each animal was housed in individual feeding pen for ease of data collection, especially during methane measurement using a laser methane detector. Each single pen had its own water trough to enable animals to have *ad libitum* access to fresh clean water. Water troughs were cleaned every morning. Feeding pens were also cleaned every morning and when necessary to avoid accumulation of faeces, which can make the feedlot environment unhygienic for animals.

3.4 Experimental design

The study was conducted as a 3 x 3 x 2 factorial experiment with treatments allocated in a completely randomised blocked design, where 3 is the diet treatments, 3 for number of animals in each group and 2 is for animal breeds. Three treatment diets with different crude fibre levels (diet 1 = 41.54 % CF, diet 2 = 18.18 % CF and diet 3 = 10.77 % CF) were tested on steers of two beef cattle breeds (Bonsmara and Nguni). The diets with different fibre levels were the treatments. Each of three treatments had three animals per breed, totalling to nine animals per breed. Treatments were arranged in replicates, to ensure reliability of the study results.

3.5 Feeding management

Experimental diets are shown in Table 3.1. The diets were mixed twice a week to ensure that animals had sufficient access to fresh feed always. Easily accessible and affordable feed ingredients were used during formulation and mixing of experimental diets (Table 3.1). The feeding of animals and collection of data were done for 90 consecutive days. The steers were given measured feed quality per individual animal and the orts were collected every morning.

Table 3.1. Ingredients used in mixing of experimental diets

Ingredients	Diet 1 (kg) (High fibre)	Diet 2 (kg) (Moderate fibre)	Diet 3 (kg) (Low fibre)
Eragrostis curvula hay	100	75	30
Sunflower oil	-	15	5
Hominy chop	45	175	300
Wheat bran	100	75	95
Cotton oil cake	25	40	20
Molasses	50	50	50
Lucerne	150	50	25
Feed lime	1	2.5	2.5
Urea	1.5	2	1.75
Salt	2.5	2.5	2.5
Premix	1.25	1.25	1.25

Eragrostis curvula hay, hominy chop and wheat bran were used during formulation as source of fibre. Molasses meal was used as source of energy, for pelleting and palatability of the diets. Cotton oil cake, urea and lucerne (*Medicago sativa*) were the source of protein. Salt and premix were utilised as source of minerals. Feed lime and sunflower oil were used as the buffering ingredient and energy balance, respectively.

3.5.2 Chemical analyses

Dry matter content of experimental diets was determined by drying fresh samples of each diet at 60 ^{o}C for 48 hours, using a technique of AOAC (ID 93.01, 2010). Experimental diets were ground to pass through a 1 mm screen for chemical analyses: Crude protein (CP), organic matter (OM) and the ether extract (EE) were determined according to the procedure of AOAC (2010). Acid detergent fibre (ADF), nutrient detergent fibre (NDF) and acid detergent lignin (ADL) and crude fibre (CF) were determined (Table 3.2) following the technique prescribed by Van Soest, Robertson & Lewis (1991). The gross energy (GE) level in diets was determined using a bomb calorimeter. For phosphorus (P) and calcium (Ca) determination, the feed samples were analysed using a method prescribed by West *et al*. (2013).

Table 3.2. Nutrient composition of experimental diets

Nutrient	Units	Diet 1 (Higher fibre)	Diet 2 (Moderate fibre)	Diet 3 (Low fibre)
DM	%	92.46[a]	91.86[b]	89.95[c]
OM	%	86.07[c]	87.34[b]	87.93[a]
CP	%	7.70[c]	10.07[a]	8.93[b]
EE	%	1.49[c]	4.21[a]	3.37[b]
GE	MJ/kg DM	16.44[c]	17.06[a]	16.95[b]
CF	%	41.54[a]	18.18[b]	10.77[c]
ADF	%	46.31[a]	16.77[b]	12.15[c]
ADL	%	11.67[c]	13.87[a]	13.65[b]
NDF	%	64.27[a]	40.44[b]	29.92[c]
Ca	%	0.47[a]	0.47[a]	0.36[b]
P	%	0.21[a]	0.21[a]	0.19[a]

Means with the different superscripts in a row are significantly different ($P < 0.05$).

DM: Dry Matter, OM: Organic Matter, CP: Crude Protein, EE: Ether Extract, GE: Gross Energy, CF: Crude Fibre, ADF: Acid Detergent Fibre, ADL: Acid Detergent Lignin, NDF: Neutral Detergent Fibre, Ca: Calcium, P: Phosphorus.

3.6 Determining growth performance of beef steers fed experimental diets

On arrival at the feedlot, animals were fed *E. curvula* hay on *ad libitum* basis for 14 days for acclimatization to the feedlot environment. Animals were gradually adapted to experimental diets to prevent ruminal acidosis and bloat (Klieve *et al*., 2003). Animals were allocated randomly to experimental diets for a period of 90 consecutive days (September to November 2015).

3.6.1 Average feed intake by animals

Animals were provided with feed equivalent to 2.5 % of their live bodyweight (LBW) per day on a dry matter basis (Formula = LBW x 0.025). The quantity of feed refusals of the previous day was recorded every morning to determine average daily feed intake (ADFI). Formula for determining average daily feed intake (ADFI) = Total feed intake – Refusals / Days on test.

3.6.2 Average daily gain (ADG)

Animals were weighed individually on arrival using cattle weighing scale (one decimal places), to determine their initial live body weight. Thereafter, animals were weighed fortnightly until the end of the study to monitor their growth rate. The mean of growth rate was calculated per group of animals per treatment and per breed. The initial body weight (IBW) was subtracted from the final body weight (FBW) to determine the weight gained during the entire feeding trial. The body weight gained (BWG) over 90 days was divided by the number of days spent by animals on the treatment diets, to compute average daily weight gain. Formula for determining average daily gain (ADG) = FBW - IBW / Days on test.

3.6.3 Feed conversion ratio (FCR).

The daily feed intake was divided by average daily gain to determine the amount of feed used by animals to gain weight during the experiment. Formula for determining FCR = ADFI/ADG.

3.7 Methane emissions determination from beef steers fed experimental diets

A hand-held laser methane detector was used to measure the concentration of methane exhaled and belched out by each animal early morning (08:30), at a distance of 3 m away from the animal, during feeding. A red beam from laser methane detector was pointed at the muzzle of each animal and readings were recorded at 5 second intervals for a period of one minute

(Chagunda, 2013). The recording of 1 minute were repeated four times a day and this was conducted for five consecutive days for each animal. During this period, the animals were fed only ground *E. curvula* hay. Grinding of hay reduced wastage of hay that is usually experienced if animals are allowed to feed on a raw hay bale. Methane concentrations were measured after the animals had been adapted to experimental diets over a period of 30 days.

3.8 Collection of rumen fluid samples

3.8.1 Method for collection of rumen fluid samples

The preparation of bottles for rumen fluid samples collection by ARC-API standard operating procedure number: SR 007. A qualified, registered and experienced animal health technician performed the entire process of rumen fluid sample collection. Rinsing of oesophageal tube was done before and after every ruminal collection from each animal with distilled water to prevent infections and sample contamination. An oesophageal tube was gently inserted into the mouth; it passed through oesophagus and smoothly pushed down until it reached rumen (Geishauser, 1993). The rumen fluid collection process was done as fast as possible to ensure that the samples reached the laboratory as fresh as possible and to minimise exposure to aerobic conditions.

3.9 Rumen pH determination from beef steers fed experimental diets

Fresh rumen fluid samples were then transferred into the anaerobic cabinet to ensure that rumen fluid samples were not exposed to oxygen, since they are strictly anaerobic. Total microbial count procedure was carried out using the same samples under anaerobic conditions. A pH – meter was used to measure rumen pH. Distilled water was used to rinse the glass electrode part of a pH meter. This was done to ensure that the device is clean before inserting it into the next sample.

3.10 Rumen total microbial count determination from beef steers fed experimental diets

3.10.1 Anaerobic microbiological procedures

The anaerobic cabinet with controlled environment was used as a platform to perform all inoculation procedures from the beginning until the point where samples were ready to be incubated. The atmospheric composition was made up of 30 % CO_2, 5 % hydrogen and 5 % nitrogen gas for balance. An anaerogen was used to keep anaerobic condition during incubation of samples at 39 ^{o}C for 48 hours. Once the petri dishes have been packed inside the incubation jars, a pack of anaerogen was inserted before closing each jar. The jars were transferred to normal incubators for a period of 48 hours. Anaerogen is capable of maintaining the anaerobic condition within a jar for 36 hours (Imhof & Heinzer, 1996).

3.10.2 Preparation of anoxic diluent

The methodology for preparation of anoxic diluent was adapted from Caldwell and Bryant (1966). While stirring, 1360.5 ml of distilled water, 37.5 ml of mineral solution A, 37.5 ml of mineral solution B and 1.5 ml of indigo carmine (0.5 % m/v) were added in a 2 litre Schott bottle containing a teflon-coated magnetic stirrer bar. The Schott bottle was then fitted with 4-port lid, which had a dip tube attached to the lower end of one port and a gas vent filter connected to the Luer-lock fitting of another port. Except for the gas vent port, the ports were then plugged with Luer male plugs. The solution was sterilised together with a bottle-top dispenser at 121 ^{o}C for 25 min. After sterilization, the sterile gas inlet filter was attached to the supply of oxygen-free gas mixture and plugged into the port carrying the dip tube.

The solution was cooled down to approximately 50 ^{o}C. Sterile 60 ml of $NaHCO_3$ (6.28 %), cysteine, 3 ml of $HCl.H_2O$ (12.5 % m/v) and 3ml of $Na_2S.9H_2O$ (12.5 % m/v) were injected through one of the ports, aseptically. While stirring, the solution was gassed with oxygen-free

gas mixture for 15 min. All ports were then sealed, disconnected from gas supply and transferred to the anaerobic cabinet together with dispenser. Inside anaerobic cabinet, lid for dispenser top was exchanged. Diluent was allowed to reduce to very light-yellow colour. Nine ml volumes / tube of the anaerobic diluent was dispensed into sterile test tubes. In this form, the anaerobic diluent was ready to be mixed with 1 ml volume of fresh rumen fluid sample.

3.10.3 Composition of 1 litre vitamin solution

A solution made up of 10 mg of *p*-amino benzoic acid, 10 mg of nicotinic acid, 10 mg of calcium pantothenate, 10 mg of pyridoxine, 10 mg of riboflavin, 10 mg of thiamine, 5 mg of biotin, 5 mg of folic acid, 5 mg of lipoic acid and 5 mg of vitamin B12 was used. Then 10 ml of this solution was added to 1 litre of anoxic medium. The final amounts used depend on the amount of anoxic medium needed.

3.10.4 Preparation of microbial growth medium (500 ml)

The methodology for preparation of De Man, Rogosa and Sharpe (MRS) agar medium was adapted from Caldwell and Bryant (1966). The 500 ml of distilled water (H_2O), 33.6 g of MRS agar were mixed together and autoclaved for 15 min in a 1 litre Schott bottle fitted with a port bottle top. After sterilization the sterile gas inlet filter was attached to the supply of oxygen free gas mixture and plugged into the port carrying the dip tube. The solution was transferred to a water bath and cooled down to approximately 50 ^{o}C. One ml of L-cysteine solution was added aseptically. Ports were closed, and the solution was transferred to the anaerobic cabinet.

3.10.5 Determining rumen total microbial count

The method for determining rumen microbial count was adapted from Mahadevan *et al*. (1982). One ml volume of fresh rumen fluid was added to 9ml volumes of anaerobic diluent. The process continued with ten-fold dilution of the original sample and diluted up to E08. One ml volumes of the dilutions E06, E07 and E08 was placed into labelled, sterile disposable petri

dishes. Same procedure was done to both inoculated petri dish and appropriately labelled control dish containing no sample and 1ml un-inoculated diluent for each sample. Fifteen ml per plate of MRS agar solution was added as a growth media. The contents of the dishes were then swirled to achieve thorough mixing. The agar was allowed to set.

The dishes were inverted and stacked in transparent plastic canisters to protect the contents against desiccation. Incubation at 39^{o}C in oxygen-free atmosphere took place for 48 hours. Control dishes were examined for possible contamination. If more than colony is observed in these dishes, the whole counting procedure would be rejected, using fresh diluent or medium, as the case may be. The colonies on each inoculated petri dish containing between 30 and 300 colonies were counted. The figures were then multiplied by the dilution factor to obtain colony-forming units (cfu) per gram / ml of sample. The average count per sample was then counted.

3.11 Determining rumen methanogenic *archaea* counts

The entire method for determining rumen methanogenic *archaea* counts was adapted from Carberry *et al*. (2014). The DNA extraction was done from rumen fluid, according to the DNA extraction kit manufacturer instructions. Universal primers were used to detect rumen *archaea* counts, using a real time PCR molecular method. Quantification of methanogenic *archaea* counts was performed by quantitative real-time PCR (qRT-PCR) analysis of the 16S rRNA genes. This part was done at the Biotechnology Platform section of the Onderstepoort Veterinary Institute - Agricultural Research Council, South Africa.

3.12 Data analysis

Shapiro-Wilk's test was performed to test for normality. Data on growth parameters (feed intake, ADG and FCR), rumen parameters (pH, total microbial count and methanogenic *archaea* count) and methane emission were analyzed by Analysis of Variance (ANOVA) using General Linear Models (GLM) procedure of SAS version 9.2 (SAS, 2008). Student's t-LSD (least significant difference) was calculated at the 5 % level ($P < 0.05$) to compare treatment means for significant effects, using the PDIFF option of SAS. The statistical models used were as follows:

(1) $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where: Y_{ij} = observation, μ = population mean constant common to all observations, T_i = effect of diet, and ε_{ij} = random error term.

(2) $Y_{ijk} = \mu + T_i + B_j + (T*B)_{ij} + \varepsilon_{ijk}$, where: Y_{ijk} = observation, μ = population mean constant common to the observation, T_i = effect of diet, B_j = effect of animal breed, $T*B_{ij}$ = effect of diet interacting with breed and ε_{ij} = random error term.

(3) $Y_{ijk} = \mu + T_i + B_j + b_1X_1 + b_2X_2 + b_3X_3 + \varepsilon_{ijk}$, where: Y_{ijk} = observation (methane emission), μ = population mean constant common to the observation, T_i = effect of diet, B_j = effect of animal breed, b_1X_1 = effect of rumen pH, b_2X_2 = effect of rumen total microbial counts, b_3X_3 = effect of rumen *archaea* counts, and ε_{ijk} = random error term.

Chapter 4

4 Results and discussions

4.1 Growth performance of Bonsmara and Nguni steers fed experimental diets

4.1.1 Daily feed intake

Daily feed intake of diet 1 was significantly lower ($P < 0.05$) than that of diet 2 within breeds (Table 4.1). This was due to high fibre content (41.54 % CF) on diet 1. These results are consistent with the findings of Wieland (2002), who reported that consumption of high fibre diets is reduced by slow feed passage through the digestive system of an animal. This means that a high fibre diet will be less digestible with consequent reduction in daily feed intake.

Table 4.1. Daily feed intake for beef steers fed experimental diets

Parameter	Breed	Diet 1 (High fibre)	Diet 2 (Moderate fibre)	Diet 3 (Low fibre)
DFI (kg / day)	Bonsmara	$8.8^{bc} \pm 0.66$	$10.7^{a} \pm 0.78$	$10.0^{ab} \pm 0.50$
	Nguni	$6.5^{e} \pm 0.99$	$8.0^{a} \pm 0.90$	$6.9^{de} \pm 0.56$
P-value	<0.0001	0.006		

Means with the different superscripts in a row are significantly different ($P < 0.05$).

DFI: Daily feed intake, kg / day: kilogram per day.

Breed factor had significant effects on daily feed intake of the experimental animals, specifically for diet 1 and diet 3. Bonsmara had significantly higher DFI ($P < 0.05$) on diet 1 and diet 3 than Nguni on the same diets, except for diet 2 (Table 4.1). This was partially due to the fact that Bonsmara is a medium frame, whereas Nguni is a small frame. Therefore, Bonsmara is expected to consume more than Nguni. These results are supported by Mukuahima (2005), who described Bonsmara as a medium frame and Nguni as a small frame breed.

4.1.2 Average daily gain

Highest average daily gain was observed in steers fed diet 3 for both breeds (Figure 4.1). This was due to low fibre content (10.77 % CF) in diet 3. These results are consistent with findings of Brondani *et al*. (2004) who observed an increase in average daily gain of beef steers finished in feedlot when the fibre content was reduced in the diet. This shows that in addition to reducing feed intake and increasing methane emission, high fibre diet will also reduce digestion and nutrient absorption. This is supported by Van Soest (1994), who stated that NDF is the cell wall proportion that reduce both the intake and digestibility of the roughage.

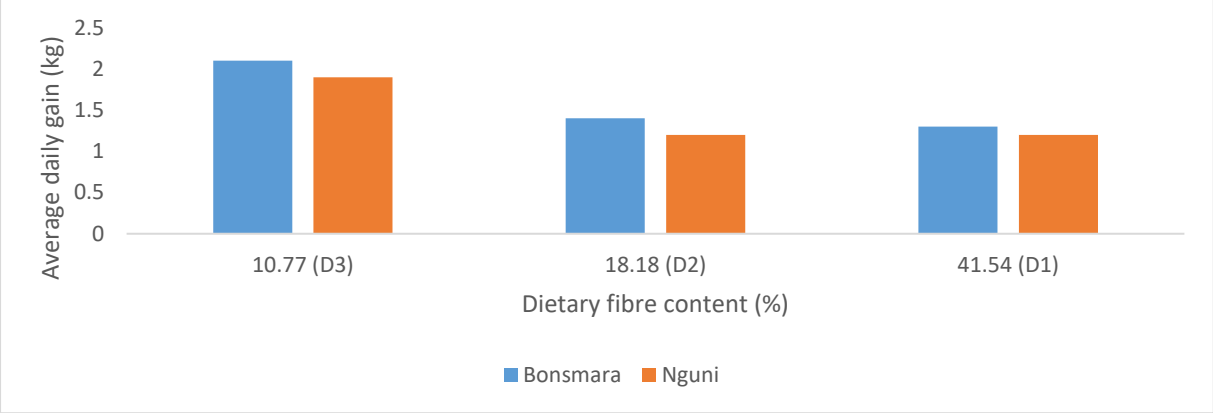

Figure 4.1. Effects of dietary fibre level on average daily gain of beef steers

Bonsmara had the higher average daily gain compared to the Nguni for all treatment diets (Figure 4.1), although not significantly different ($P > 0.05$). This shows that Bonsmara breed is genetically better than Nguni breed as far as average daily gain is concerned. These findings are consistent with the findings of Linde, Scholtz & Marle-Köster (2018), whose results showed that average daily gain of Bonsmara was higher than that of Nguni, for both low and high fibre diets. Nguni had similar average daily gain for diet 1 and 2, irrespective of the differences in dietary fibre levels. This shows the ability of Nguni cattle to efficiently utilize high fibre diets.

4.1.3 Feed conversion ratio

Feed conversion ratio of Nguni breed was significantly lower ($P < 0.05$) than that of a Bonsmara breed for diet 1 and diet 2 (Table 4.2), except for diet 3. This shows that on average, Bonsmara used approximately 6.8 kg of high fibre (41.54 % CF) diet 1 to gain 1 kg of live body mass (6.8:1). These results are consistent with the findings of Muchenje *et al*. (2008) who reported that the Bonsmara is not as well adapted to harsh conditions as the Nguni, although it compete with other European beef cattle breeds under subtropical conditions.

Table 4.2. Feed conversion ratio of Nguni and Bonsmara steers fed experimental diets

Parameter	Breed	Diet 1 (High fibre)	Diet 2 (Moderate fibre)	Diet 3 (Low fibre)
FCR	Bonsmara	$6.8^{abc} \pm 0.96$	$8.1^{cd} \pm 0.96$	$4.8^{bcd} \pm 0.56$
	Nguni	$5.4^{cd} \pm 0.27$	$7.1^{ab} \pm 0.86$	$3.8^{d} \pm 0.97$
P-value	0.069	0.005		

Means with the different superscripts in a row are significantly different ($P < 0.05$).

FCR: Feed conversion ratio.

On a contrary, Nguni cattle used 5 kg of same high fibre (41.54 % CF) diet 1 to gain 1 kg of live body mass (5:1). These results are consistent with the findings of Collins-Luswet (2000) who reported that the indigenous Nguni cattle breed of South Africa is adapted to harsh environments. Nguni was able to efficiently convert the less amount (6.5 kg) of fibrous Diet 1 into a reasonable weight gain (1.2 kg). These results are supported by Linde, Scholtz and Marle-Köster (2018), who reported that FCR of Nguni improved when steers were fed high fibre diets. This was due to intramuscular fat development genes that were highly expressed in Nguni cattle fed high fibre diet (Linde, Scholtz & Marle-Köster, 2018). Feed conversion ratio of diet 3 was significantly lower ($P < 0.05$) than that of diet 2 for Nguni cattle. This was due to low fibre in diet 3.

4.2 Rumen pH, rumen total microbial count, rumen methanogenic *archaea* count and methane emissions for beef steers fed experimental diets

4.2.1 Rumen pH

An increase in rumen pH was observed (Figure 4.2) when the dietary fibre content was increased, although there was no significant difference ($P > 0.05$) observed both within and between cattle breeds. These results are supported by Dado and Allen (1993) who reported the use of physical effective fibre as the most viable alternative for enhancing salivary buffer supply to maintain rumen pH. This also results in greater ruminal fermentation and microbial protein synthesis and consequently benefit the host animal by improving its rumen functioning.

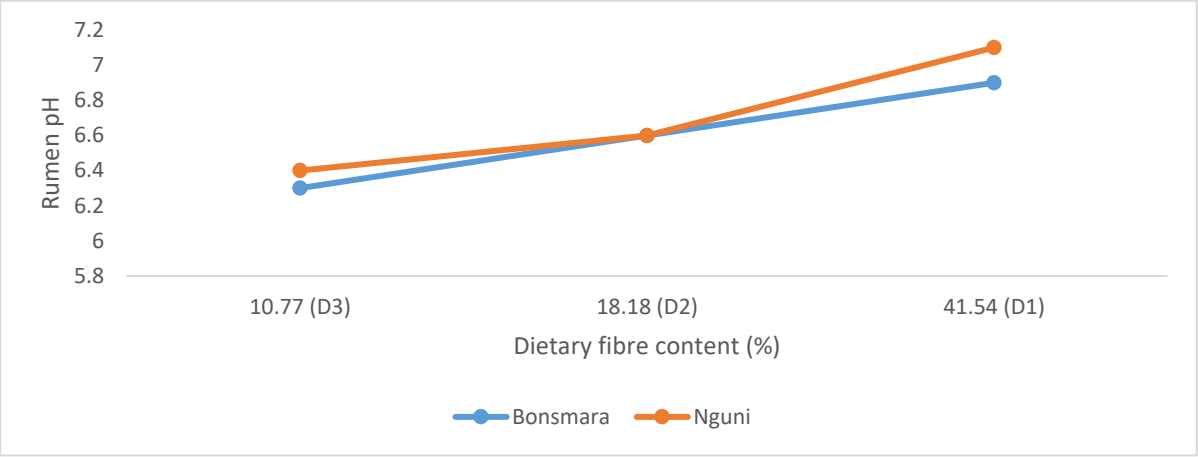

Figure 4.2. Relationship between dietary fibre content and rumen pH

Brown, Ponce and Pulikanti (2006) who reported that enteric fermentation of high fibre diet results in balanced microbial activity from ingestion until the excretion point with a normal rumen pH (6 to 7). This was observed in diet 1 (Figure 4.2). Moderate fibre content in diet 2 resulted to moderate rumen pH for both Bonsmara and Nguni cattle breeds ($P > 0.05$). Steers on diet 3 did not have excessive drop in rumen pH, indicating that 10.77 % crude fibre resulted in adequate buffering capacity. Low rumen pH in diet 3 steers is consistent with Dado and Allen (1993) who observed a rapid decline in rumen pH following a decrease in dietary fibre.

4.2.2 Rumen total microbial count

High rumen total microbial count serves as a strong indication of good rumen functioning in domesticated ruminants (La Reau & Suen, 2018). The results of this study on Table 4.3 shows exactly that. Rumen total microbial count in low fibre (diet 3) was significantly higher ($P < 0.05$) than diet 1 and 2 (Table 4.3) for both breeds. This should be a reason for high ADG by steers fed diet 3, which was 2.1 kg/day for Bonsmara and 1.9 kg/day for Nguni (Figure 4.1), and significantly better FCR ($P < 0.05$). There was no significant difference ($P > 0.05$) between high fibre (diet 1) and moderate fibre (diet 2).

Table 4.3. Rumen total microbial count of beef steers fed experimental diets

Parameter	Breed	Diet 1 (High fibre)	Diet 2 (Moderate fibre)	Diet 3 (Low fibre)
RTMC (cfu / ml)	Bonsmara	$1.23x10^{6c} \pm 0.98$	$3.85x10^{6c} \pm 0.65$	$9.3x10^{7a} \pm 0.12$
	Nguni	$5.16x10^{6c} \pm 0.66$	$5.63x10^{6c} \pm 0.40$	$3.15x10^{7b} \pm 0.78$
P-value	<0.0001	<0.0001		

Means with different superscripts in a row are significantly different ($P < 0.05$).

RTMC: Rumen total microbial count, cfu / ml: colony forming units per millilitre.

These results are concurring with the findings of Guan *et al*. (2008) who stated that presence of particular rumen microbes is regulated by the interaction between animal genetic make-up and diet composition, which subsequently affects average daily gain. Rumen total microbial count in low fibre diet (diet 3) was also significantly different ($P < 0.001$) between Bonsmara and Nguni cattle breeds. There was no significant difference ($P > 0.05$) between breeds fed a low fibre (diet 1) and moderate fibre (diet 2).

4.2.3 Rumen methanogenic *archaea* count

Low rumen methanogenic *archaea* count was observed (Figure 4.3) in Bonsmara and Nguni steers fed on diet 3 (low fibre), although not significantly different ($P > 0.05$). This was consequently associated with low rumen pH, which was also not significantly different ($P > 0.05$). These results are supported by observations of Hünerberg *et al*. (2015), who reported that methanogenic *archaea* are sensitive to low rumen pH levels. This also corresponds well with good FCR that was observed (Table 4.2) in steers on diet 3, irrespective of cattle breed. Good FCR indicates that the majority of ingested feed was used by the host animal to gain body weight, rather than being used by rumen methanogenic *archaea* to produce methane.

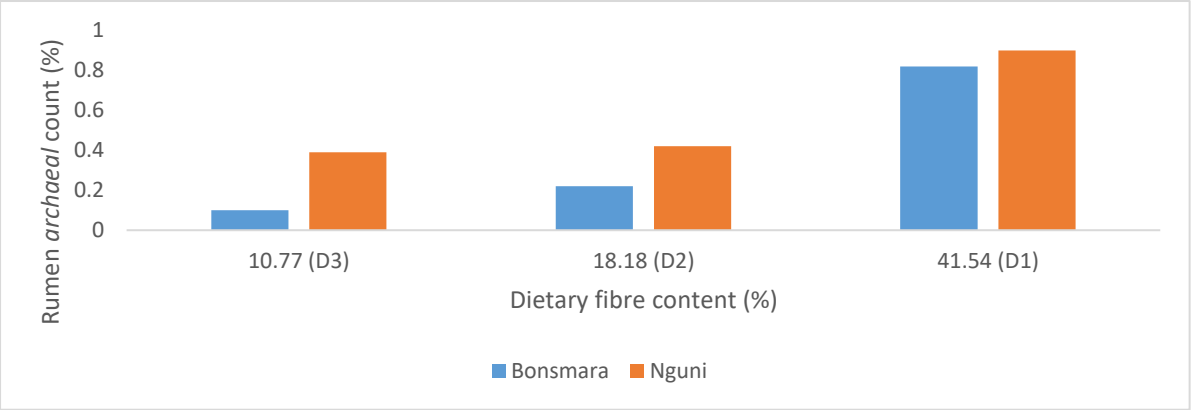

Figure 4.3. Effects of dietary fibre level on rumen *archaeal* counts

High rumen methanogenic *archaea* count was observed (Figure 4.3) in steers fed diet 1 (high fibre). These results are supported by Kamra (2005), who stated that methanogenic *archaea* populations present in the rumen depend on the diet consumed by the host animal. Low rumen *archaea* count was observed in Bonsmara breed (Figure 4.3) than in Nguni breed across all treatment diets, which positively correlated with average daily gain of the respective diets and cattle breeds. This was due to the good feed efficiency of Bonsmara breed. These results are consistent with findings of Whitford *et al*. (2001), who reported that rumen methanogenic *archaea* count is associated with the feed efficiency of a host animal.

4.2.4 Methane emissions

Low methane emissions were observed in diet 3 for both cattle breeds (Table 4.4). This was influenced by low fibre level in diet 3 (Table 3.2). These results are consistent with the findings of Sauvant and Giger-Reverdin (2007) who observed a decrease in methane production for ruminants that were fed high concentrate diets. Diet 3 (low fibre) was able to reduce methane emission while improving growth performance of Bonsmara and Nguni steers, compared to other two treatment diets.

Table 4.4. Methane emissions from Bonsmara and Nguni steers fed experimental diets

Parameter	Breed	Diet 1 (High fibre)	Diet 2 (Moderate fibre)	Diet 3 (Low fibre)
CH_4 (ppm / m)	Bonsmara	$1513^{a} \pm 0.08$	$851^{ab} \pm 0.01$	$655^{b} \pm 0.61$
	Nguni	$1604^{a} \pm 0.10$	$972^{ab} \pm 0.06$	$927^{ab} \pm 0.85$
P-value $_{(0.05)}$	0.445	0.020		

Means with different superscripts in both rows and columns are significantly different ($P < 0.05$).

CH_4: Methane emission, ppm / m: parts per million per minutes.

High methane production in Diet 1 for both breeds (Table 4.4) was due to high crude fibre level in diet 1 (Table 3.2). These results are supported by the findings of Hindrichsen *et al*. (2006), who reported that methanogens produce more methane when the host animal is fed high fibrous feed. Poor average daily gains observed (Figure 4.1) in steers fed diet 1 indicates that the majority of ingested feed was utilised by rumen methanogenic *archaea* to produce methane (Table 4.4) that was higher in diet 1 compared to diet 3 and 2, rather than being used by the host animal to gain weight. This was further indicated by poor FCR that observed in steers fed diet 1, irrespective of cattle breed.

4.3 Correlation between measured parameters

4.3.1 Impact of rumen pH of methane emission

There was a strongly positive correlation (R^2 = 0.9105) between rumen pH and methane emission for Bonsmara steers (Figure 4.4). An increase in rumen pH resulted in an increase of methane emission for Bonsmara steers. These results are consistent with the findings of Hünerberg *et al*. (2015), who observed an increase in methane production when rumen pH was increased.

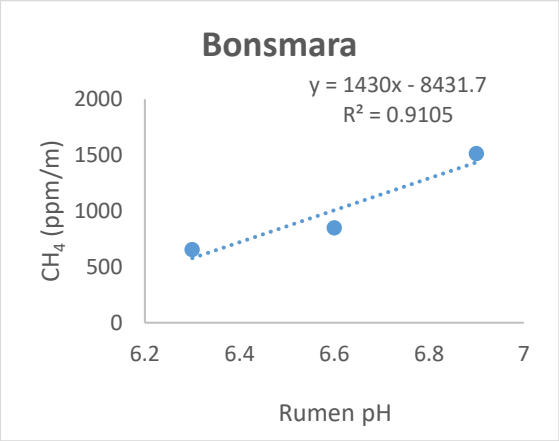

Figure 4.4. Effects of rumen pH on CH_4

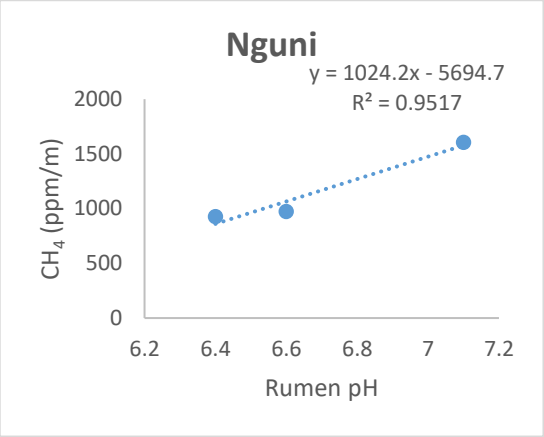

Figure 4.5. Effects of rumen pH on CH_4

A highly positive correlation (R^2 = 0.9517) was also observed between methane emission and rumen pH for Nguni steers (Figure 4.5). This shows that beef cattle can be fed low fibre diets to reduce their rumen pH, and consequently reduce methane emission. Such intervention would reduce methane emitted by beef cattle, and thus reduce contribution of ruminants towards global warming.

4.3.2 Impact of rumen total microbial count on feed conversion ratio

A negative correlation (R^2 = 0.8286) was observed between rumen total microbial count and feed conversion ratio for Bonsmara steers (Figure 4.6). An increase in rumen total microbial count resulted in a remarkable reduction of feed conversion ratio. These results are supported by Hernandez-Sanabria *et al.* (2011), who reported that rumen microbes are responsible for enteric fermentation and consequently determine the feed efficiency.

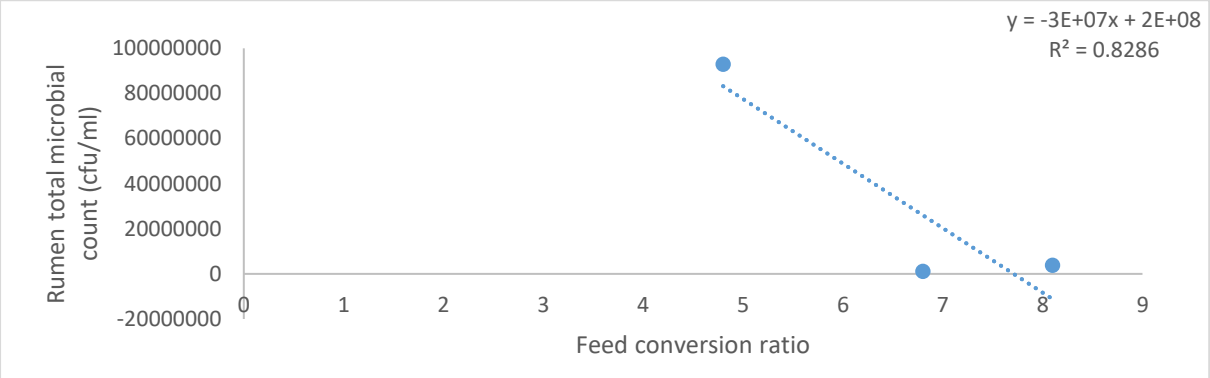

Figure 4.6.: Effects of rumen total microbial count on FCR for Bonsmara steers

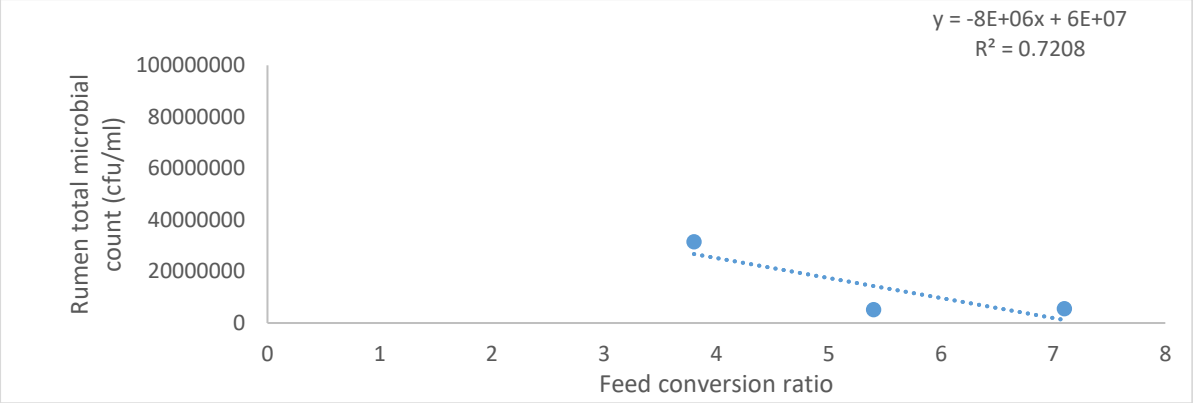

Figure 4.7. Effects of rumen total microbial count on FCR for Nguni steers

This was due to good rumen fermentation, which is a result of high rumen microbial count. There was a negative correlation (R^2 = 0.7208) between rumen total microbial count and feed conversion ratio for Nguni steers (Figure 4.7).

Chapter 5

5 Conclusion, recommendations and scope for further research

5.1 Conclusions

Feeding a diet that is low in crude fibre improved growth performance (daily feed intake, average daily gain and feed conversion ratio) of both Bonsmara and Nguni steers. On a contrary, feeding a diet with high crude fibre had reduced growth performance of both Bonsmara and Nguni steers, which confirms what most studies have found out. Bonsmara breed had the highest feed intake across all diets compared to Nguni breed. However, Nguni breed had a better feed conversion ratio when fed high fibre diets. This points to the difference, which may exist in feed efficiency of these two breeds.

Feeding low fibre diet to Bonsmara and Nguni steers had reduced rumen pH due to lactic acid accumulation in the rumen, caused by a shift in microbial populations from gram-negative predominance to gram-positive lactic acid producers. This did not result in excessive drop of rumen pH in both breeds. Feeding a diet that is low in crude fibre had reduced rumen *archaea* counts, and consequently reduced methane production. Treatment diet with 10.77 % crude fibre level managed to reduce methane production while improving growth performance of Bonsmara and Nguni steers, when compared with other treatment diets. Bonsmara breed had the lowest rumen *archaea* count across all treatments, due to its good feed efficiency. This could be related to high intake and possible increased rate of passage through the gut on top of genetic variation.

5.2 Recommendations

Feeding diets with low fibre levels is recommended for improving rumen total microbial count of Bonsmara and Nguni steers, which is actually a sign of proper rumen functioning. High

rumen total microbial count will consequently result in improved growth performance of Bonsmara steers, due to efficient rumen fermentation and feed absorption. However, the findings of this study, supported by recent literature, show that Nguni steers perform better in terms of feed conversion ratio even when fed high fibre diets. It is recommended that the genetic potential (dietary fibre utilisation) of cattle breed should be understood and considered when formulating diets for beef cattle.

Low dietary fibre inclusion level can be used as a strategy to keep rumen pH at low level, which will result in reduced rumen *archaea* counts in beef cattle and consequently lower methane emitted by the host animal. Results of this study, supported by the literature, show the above mentioned can be used as a viable strategy to reduce contribution of ruminants, especially beef cattle, towards global warming. The findings of this study, supported by literature, showed that Bonsmara emitted less methane compared with Nguni for all treatment diets. The use of feed efficient cattle breeds that harbour less *archaea spp* such as Bonsmara can be applied and advocated as an alternative climate smart beef farming strategy.

5.3 Scope for further research

The findings of this study indicated low dietary fibre (concentrates) inclusion and use of feed efficient cattle breed as the solutions for reducing methane production in beef cattle, without compromising growth performance. However, high cost of concentrates might make it difficult for under resourced beef farmers to implement the findings of the current study. Therefore, another study is recommended for further research on nutritious, easily accessible fodder plant that can reduce methane production in beef cattle at a reduced price.

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